Glucose OF Medium (NCM0152)

Intended Use
Glucose OF Medium is used for the confirmation of Enterobacteriaceae in foods according to ISO 21528-1&2:2017, and is not intended for use in the diagnosis of disease or other conditions in humans.

Description
Enzymatic digest of casein provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dipotassium phosphate acts as a buffer system. Glucose is the fermentable carbohydrate providing carbon and energy. Bromothymol blue is the pH indicator.

Colonies of presumptive Enterobacteriaceae should be confirmed by means of tests for the fermentation of glucose and the presence of oxidase. If the colonies are oxidase-negative and glucose-positive, the sample shall be regarded as being positive for Enterobacteriaceae. ISO 21528 :2017 recommends this medium to confirm the presumptive colonies of Enterobacteriaceae by fermentation test.

Typical Formulation

Enzymatic Digest of Casein	2.0 g/L
Dipotassium Hydrogen Phosphate	0.3 g/L
Glucose	10.0 g/L
Sodium Chloride	5.0 g/L
Bromthymol Blue	0.08 g/L
Agar	4.0 g/L

Final pH: 6.8 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
1. Refer to SDS

Preparation
1. Dissolve 21.4 grams of the medium into one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium if necessary.
3. Autoclave at 121ºC for 15 minutes.
4. Cool to 45-50ºC.

Test Procedure
ISO 21528-1: For the detection and enumeration of Enterobacteriaceae in foods using the MPN technique:

This requires test samples to be added to 90ml and 9ml of Buffered Peptone Water (NCM0015) in a 1:9 ratio. After incubation of nine tubes at 37ºC for 16-20 hours, each broth is streaked onto VRBGA (NCM0041) and incubated at 37ºC for 22-26 hours. Characteristic colonies are then streaked onto Nutrient Agar (NCM0033) and confirmed by the oxidase reaction and the fermentation of glucose (using Glucose OF Medium, NCM0152). For the fermentation test, using a sterile wire stab the same colonies which gave a negative oxidase test into tubes containing 10ml Glucose OF Medium. Overlay the surface with at least 1cm of sterile mineral oil before incubating at 37ºC for 22-26 hours. A positive reaction is indicated by a color change in the agar from green to yellow.

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Technical Specification Sheet

ISO 21528-2: For the detection and enumeration of Enterobacteriaceae in foods using the colony-count technique:

Prepare a single decimal dilution series from the test sample if the product is liquid, or from the initial suspension in the case of other products. Using a sterile pipette transfer 1ml of the test sample to a Petri dish. Repeat with further dilutions if necessary. Add approximately 15ml of VRBGA (NCM0041) to each dish, allow to cool and invert before incubating at 37°C for 22-26 hours. Count colonies in dishes with less 150 characteristic colonies. Subculture five characteristic colonies on a non-selective agar such as Nutrient Agar (NCM0033) before confirming by the oxidase reaction and the fermentation of glucose (using Glucose OF Medium, NCM0152). For the fermentation test, using a sterile wire stab the same colonies which gave a negative oxidase test into tubes containing 10ml Glucose OF Medium. Overlay the surface with at least 1cm of sterile mineral oil before incubating at 37°C for 22-26 hours. A positive reaction is indicated by a color change in the agar from green to yellow.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige with a green tint.

Prepared Appearance: Prepared medium is a green gel.

Minimum QC:

Salmonella typhimurium ATCC 14028	Good Growth, Blue/Green Colonies
Salmonella enteritidis ATCC 13076	Good Growth, Blue/Green Colonies
Enterobacter aerogenes ATCC 13048	Growth, Black Colonies
Pseudomonas aeruginosa ATCC 27853	Inhibited

Results

Growth Characteristics		
Organism	**Growth**	**Color**
Salmonella spp.	Good Growth	Blue/Green
Enterobacter spp. *Klebsiella* spp	Growth	Black
Escherichia coli	Suppressed	Colorless
Shigella spp.	Suppressed	Colorless
Proteus spp.	Suppressed	Colorless to Brown

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

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Storage

Store dehydrated culture media at 2-25°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 21528-1:2017 Microbiology of the food chain– Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 1: Detection of Enterobacteriaceae.
2. ISO 21528-2:2017 Microbiology of the food chain– Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony-count technique.

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